

Vitamin K₅ as a Fungistatic Agent¹

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ABSTRACT

MERRIFIELD, LARRY S. (Oregon State University, Corvallis), AND H. Y. YANG. Vitamin K₅ as a fungistatic agent. Appl. Microbiol. 13:660-662. 1965.—The effectiveness of vitamin K₅ in controlling the growth of different molds at varying pH levels in a culture medium, in tomato juice, and in several berry purees was studied. The molds studied were *Aspergillus*, *Botrytis*, *Hormodendrum*, *Mucor*, and *Penicillium*. The results showed that vitamin K₅ was effective as a fungistatic agent at concentrations ranging from 0.006 to 0.02%.

Previous work has shown that vitamin K₅ (4-amino-2-methyl-1-naphthol hydrochloride), an analogue of vitamin K, has a marked inhibitory activity toward a number of microorganisms (Armstrong, Spink, and Kahnke, 1943; Pratt et al., 1950; Schwartzman, 1948).

Pratt and his co-workers (1950) were among the first to demonstrate that vitamin K₅ was an effective antifungal agent. Masuo and Okabayashi (1953) found it to be an effective fungistatic agent against several molds. Vitamin K₅ was also shown to be effective against the spores of molds (Trivellato, 1957). A similar study showing the effectiveness of sorbic acid as a fungistatic agent against specific fungi was reported by Beneke and Fabian (1955).

The fact that vitamin K₅ is being studied as a possible food preservative, along with the limited amount of work done on specific molds, led to this investigation of its fungistatic activity against a number of specific fungi.

MATERIALS AND METHODS

Five different genera of fungi isolated from foods were selected for use in the studies: *Aspergillus niger*, *Botrytis* sp., *Hormodendrum* sp., *Mucor* sp., and *Penicillium roqueforti*. The fungi were pure cultures obtained from the Department of Microbiology, Oregon State University.

The vitamin K₅ used throughout this study was synthesized according to the method of Oneto and Sah (1949).

All five organisms were used in determining the effectiveness of vitamin K₅ in Malt Extract Broth (Difco). Three different concentrations of vitamin K₅, i.e., 0.002, 0.01, and 0.02%, were tested against the fungi in the medium adjusted to pH 3, 5, and 7. The tubes containing the broth were inoculated

in duplicate, and observation for any growth that occurred was noted at the end of 7 days.

The second series of tests on the fungistatic activity of vitamin K₅ was run against the same five molds with tomato juice as a medium. Commercially canned tomato juice (pH 4.4) was obtained and sterilized in the autoclave for 15 min at 15 psi (121 C). A 20-ml amount of juice was added to a 50-ml Erlenmeyer flask, and the molds, which were inoculated into 5 ml of Malt Extract Broth and incubated at 29 C for 5 days, were added individually along with the vitamin K₅. The flasks were stoppered, and the whole mixture was shaken until thoroughly mixed. The contents of the flasks were then placed in sterile petri dishes and incubated at 29 C. The plates were observed for growth after 2, 7, and 14 days. Concentrations of vitamin K₅ utilized were 0.004, 0.008, and 0.01%, with all concentrations and a control done in duplicate.

The third, fourth, and fifth series of tests were all conducted in a similar manner with strawberries (pH 3.8), blackberries (pH 3.4), and loganberries (pH 3.1). The berries were all obtained from the Department of Food Science and Technology, Oregon State University, as fresh frozen berries, and, after thawing, they were put through a pulper and made into a puree. The puree was then sterilized in the autoclave for 15 min at 15 psi (121 C). The concentrations of vitamin K₅ used for all three kinds of berries were 0.006, 0.01, and 0.02%, and each concentration plus a control was run in duplicate.

RESULTS

All the molds grew in the Malt Extract Broth at pH 5 and 7, but only *A. niger* and *P. roqueforti* grew at pH 3 (Table 1). When vitamin K₅ at a concentration of 0.002% was added to the medium, no growth occurred for *P. roqueforti* at any pH level. *A. niger* was the only mold to grow at pH 3 with a vitamin K₅ concentration of

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TABLE 1. *Fungistatic action of vitamin K₅ at various concentrations in a Malt Extract Broth at different pH levels*

Fungus	pH	Growth at various concn of vitamin K ₅			
		Con-trol	0.002%	0.01%	0.02%
<i>Aspergillus niger</i>	3	+	+	—	—
	5	+	+	—	—
	7	+	+	—	—
<i>Botrytis</i> sp.	3	—	—	—	—
	5	+	+	—	—
	7	+	+	—	—
<i>Hormodendrum</i> sp.	3	—	—	—	—
	5	+	+	—	—
	7	+	+	—	—
<i>Mucor</i> sp.	3	—	—	—	—
	5	+	+	—	—
	7	+	+	—	—
<i>Penicillium roqueforti</i>	3	+	—	—	—
	5	+	—	—	—
	7	+	—	—	—

* Symbols: + = growth at the end of 7 days; — = no growth at the end of 7 days.

TABLE 2. *Effect of vitamin K₅ at various concentrations in a tomato juice medium at pH 4.4*

Fungus	Days	Growth* at various concn of vitamin K ₅			
		Con-trol	0.004%	0.008%	0.01%
<i>Aspergillus niger</i>	2	+	+	+	—
	7	+	+	+	+
	14	+	+	+	+
<i>Botrytis</i> sp.	2	+	+	—	—
	7	+	+	+	—
	14	+	+	+	+†
<i>Hormodendrum</i> sp.	2	+	+	—	—
	7	+	+	+	+
	14	+	+	+	+
<i>Mucor</i> sp.	2	+	+	—	—
	7	+	+	—	—
	14	+	+	+	+†
<i>Penicillium roqueforti</i>	2	+	—	—	—
	7	+	+	—	—
	14	+	+	—	—

* Symbols: + = growth; — = no growth.
† Slight growth.

0.002%, whereas all the molds except *P. roqueforti* showed growth at pH 5 and 7 and a vitamin K₅ concentration of 0.002%. There was no growth for any of the molds when the vitamin K₅ concentration was 0.01 or 0.02%, even after 14 days.

When tomato juice was used as a medium, the results varied among the different molds. *A. niger* grew, even with a vitamin K₅ concentration of 0.01%, after 7 days (Table 2). *Hormodendrum* showed some growth at 0.01% vitamin K₅ after 7 days, and *Botrytis* and *Mucor* showed some growth at 0.01% vitamin K₅ after 14 days; *P. roqueforti*, however, showed no growth at 0.008% vitamin K₅ even after 14 days.

With strawberry puree as the medium, 0.02% vitamin K₅ was effective in controlling the growth of all molds for 14 days (Table 3). For all molds, except *A. niger*, 0.01% vitamin K₅ was effective for 14 days, and, for *P. roqueforti* and *Mucor*, 0.006% vitamin K₅ was effective for 14 days.

A concentration of 0.02% vitamin K₅ was effective in controlling the growth of all the molds for 14 days in blackberry puree. All the molds except *A. niger* were held for 14 days at 0.01% vitamin K₅, and *Mucor* and *Botrytis* were held for 14 days at 0.006% vitamin K₅ (Table 4).

With loganberry puree as the medium, a concentration of 0.02% vitamin K₅ was again effective in controlling the growth of all molds for 14

TABLE 3. *Effect of vitamin K₅ at various concentrations in a strawberry medium at pH 3.8*

Fungus	Days	Growth at various concn of vitamin K ₅			
		Con-trol	0.006%	0.01%	0.02%
<i>Aspergillus niger</i>	2	+	+	—	—
	7	+	+	+	—
	14	+	+	+	—
<i>Botrytis</i> sp.	2	+	—	—	—
	7	+	—	—	—
	14	+	+	—	—
<i>Hormodendrum</i> sp.	2	+	—	—	—
	7	+	—	—	—
	14	+	+	—	—
<i>Mucor</i> sp.	2	+	—	—	—
	7	+	—	—	—
	14	+	—	—	—
<i>Penicillium roqueforti</i>	2	+	—	—	—
	7	+	—	—	—
	14	+	—	—	—

* Symbols: + = growth; — = no growth.

TABLE 4. Effect of vitamin K₅ at various concentrations in a blackberry medium at pH 3.4

Fungus	Days	Growth at various concn of vitamin K ₅			
		Control	0.006%	0.01%	0.02%
<i>Aspergillus niger</i>	2	+	+	—	—
	7	+	+	—	—
	14	+	+	+	—
<i>Botrytis</i> sp.	2	+	—	—	—
	7	+	—	—	—
	14	+	—	—	—
<i>Hormodendrum</i> sp.	2	+	—	—	—
	7	+	—	—	—
	14	+	+	—	—
<i>Mucor</i> sp.	2	+	—	—	—
	7	+	—	—	—
	14	+	—	—	—
<i>Penicillium roqueforti</i>	2	+	—	—	—
	7	+	—	—	—
	14	+	+	—	—

* Symbols: + = growth; — = no growth.

TABLE 5. Effect of vitamin K₅ at various concentrations in a loganberry medium at pH 3.1

Fungus	Days	Growth at various concn of vitamin K ₅			
		Control	0.006%	0.01%	0.02%
<i>Aspergillus niger</i>	2	+	+	—	—
	7	+	+	+	—
	14	+	+	+	—
<i>Botrytis</i> sp.	2	—	—	—	—
	7	+	—	—	—
	14	+	—	—	—
<i>Hormodendrum</i> sp.	2	—	—	—	—
	7	+	—	—	—
	14	+	—	—	—
<i>Mucor</i> sp.	2	—	—	—	—
	7	+	—	—	—
	14	+	—	—	—
<i>Penicillium roqueforti</i>	2	+	—	—	—
	7	+	+	—	—
	14	+	+	—	—

* Symbols: + = growth; — = no growth.

days (Table 5). All the molds except *A. niger* were held for 14 days with 0.01% vitamin K₅, and all except *A. niger* and *P. roqueforti* were held with 0.006% vitamin K₅ for 14 days.

DISCUSSION

The results show that there is a variation in the sensitivity of the different genera to vitamin K₅, but that it is an effective fungistatic agent even at low concentrations.

It is apparent that the pH of the medium also has an effect on its fungistatic activity. For the strawberry, blackberry, and loganberry purees, there was very little difference in the results, but the results for the tomato juice at a higher pH were quite different. A lesser concentration of vitamin K₅ is necessary for fungistatic results in the medium with the lowest pH, except for *A. niger* and *P. roqueforti*, both of which seemed to do better at the lower pH levels. In all cases, *A. niger* grows quite well, except at higher concentrations of vitamin K₅.

Beneke and Fabian (1955) used sorbic acid at a concentration range of 0.025 to 0.075% as a fungistatic agent against molds in a food medium. The highest concentration of vitamin K₅ used in this work was 0.02%, which shows that it is quite an effective antifungal agent.

The fact that vitamin K₅ in low concentrations is capable of inhibiting the growth of all the test fungi in a food medium at its natural pH suggests commercial application of it as a fungistatic agent.

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